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Biophysical basis of pituitary cell type-specific Ca^{2+} signaling–secretion coupling

Stanko S. Stojilkovic[1], Hana Zemkova[1,2] and Fredrick Van Goor[1,3]

[1]Section on Cellular Signaling, Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, MD 20892-4510, USA
[2]Institute of Physiology, Academy of Sciences of the Czech Republic, 142 20 Prague 4, Czech Republic
[3]Vertex Pharmaceuticals, San Diego, CA 92121, USA

All secretory pituitary cells exhibit spontaneous and extracellular Ca^{2+}-dependent electrical activity. Somatotrophs and lactotrophs fire plateau-bursting action potentials, which generate Ca^{2+} signals of sufficient amplitude to trigger hormone release. Gonadotrophs also fire action potentials spontaneously, but as single, high-amplitude spikes with limited ability to promote Ca^{2+} influx and secretion. However, Ca^{2+} mobilization in gonadotrophs transforms single spiking into plateau-bursting-type electrical activity and triggers secretion. Patch clamp analysis revealed that somatotrophs and lactotrophs, but not gonadotrophs, express BK (big)-type Ca^{2+}-controlled K^+ channels, activation of which is closely associated with voltage-gated Ca^{2+} influx. Conversely, pituitary gonadotrophs express SK (small)-type Ca^{2+}-activated K^+ channels that are co-localized with intracellular Ca^{2+} release sites. Activation of both channels is crucial for plateau-bursting-type rhythmic electrical activity and secretion.

Introduction

Anterior pituitary secretory cell types other than corticotrophs are derived from the same progenitor cells [1], but they differ with respect to their Ca^{2+} signaling and secretory patterns *in vivo* and *in vitro*, as illustrated by comparison of corticotrophs, lactotrophs, somatotrophs and gonadotrophs [2,3]; See Box 1. *In vivo,* removal of hypothalamo–pituitary connections leads to increased release of prolactin (PRL), but not gonadotropins. Animals bearing ectopic pituitary grafts also release high levels of PRL and low levels of luteinizing hormone (LH) for a prolonged period, leading to pseudo-pregnancy. *In vitro*, basal (in the absence of agonists) PRL and growth hormone (GH) secretion from pituitary fragments, dispersed pituitary cells and immortalized lacto-somatotrophs is high, occurs in an extracellular Ca^{2+}-dependent manner and is termed intrinsic or spontaneous secretory activity [4]. By contrast, basal LH secretion *in vitro* is low and is not affected by removal of extracellular Ca^{2+}. Because of the high levels of spontaneous GH and PRL secretion, it is not surprising that lactotrophs and somatotrophs are under negative hypothalamic control by $G_{i/o}$-coupled dopamine and somatostatin receptors, in addition to positive control by G_q- and G_s-coupled receptors [4–7].

Box 1. Patterns of electrical activity and secretion

The membrane potential (V_m) of pituitary cells is not stable but oscillates from the baseline potential of about –60 mV [35]. When V_m oscillations reach the threshold level, cells generate action potentials (APs). Depending on the amplitude and duration of V_m oscillations, cells can generate different patterns of APs. In resting gonadotrophs and several immortalized pituitary cells, V_m oscillations are small, and as yet uncharacterized pacemaker activity generates single APs that are sharp and short in duration, and reverse polarization of V_m. Consequently, they are termed single axonal-type APs. However, resting somatotrophs and lactotrophs exhibit larger V_m oscillations, on top of which the depolarizing plateau and bursts of APs are generated, with spikes that usually do not reach the reverse potential. In agonist-stimulated gonadotrophs, AP bursts are temporally synchronized with inositol (1,4,5)-trisphosphate-dependent intracellular Ca^{2+} oscillations. Such complex but organized superimposition of APs is called plateau-bursting activity [36].

APs provide an effective mechanism for rapid transmembrane and intracellular signaling. In cells expressing voltage-gated Ca^{2+} channels, APs promote extracellular Ca^{2+} influx. Both the frequency and the shape of APs encode the Ca^{2+} signal. Rapid, single APs usually generate localized Ca^{2+} signals, just below the plasma membrane, also known as domain Ca^{2+}, which cannot be detected by regular Ca^{2+} dyes. Increases in the frequency of single APs and plateau-bursting APs can generate global Ca^{2+} signals. In some cells, but not in normal pituitary cells, single APs can generate global Ca^{2+} signals by triggering the release of Ca^{2+} from ryanodine-sensitive intracellular stores. Because plateau-bursting APs are periodic in nature, this phenomenon results in oscillatory Ca^{2+} signaling [36].

In synapses, the domain Ca^{2+} is sufficient to trigger secretion of vesicles because the predocked release-ready vesicles are molecularly linked to Ca^{2+} channels localized in active zones, which facilitates their rapid release in response to single APs. Conversely, neuroendocrine and endocrine cells do not have active zones, the distance between secretory vesicles and Ca^{2+} channels is large and domain Ca^{2+} cannot reach the secretory vesicles. For example, in chromaffin cells single APs trigger only a small amount of secretion, whereas a prolonged depolarization induces massive secretion [37]. In rat melanotrophs, a short depolarization elicits only a minor degree of secretion [38]. A short depolarization of single gonadotrophs is also insufficient to stimulate exocytosis [39], in contrast to the action of high-amplitude GnRH-induced Ca^{2+} oscillations [40]. Thus, in these cells global Ca^{2+} signals are required to trigger secretion.

Corresponding author: Stojilkovic, S.S. (stankos@helix.nih.gov).
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Conversely, LH secretion from gonadotrophs is under positive hypothalamic control by Ca^{2+}-mobilizing gonadotropin-releasing hormone (GnRH) receptors [8], and only in neonatal gonadotrophs is GnRH-stimulated gonadotropin secretion inhibited by the pineal hormone melatonin [9]. Here, we review biophysical findings that help to clarify what endows lactotrophs and somatotrophs, but not gonadotrophs, with the ability to secrete in the absence of ligand stimulation.

Excitability of pituitary cells and basal secretion

Recent results obtained with perfused anterior pituitary cells indicate that spontaneous GH and PRL secretion is much higher than basal LH secretion [10]. Figure 1a compares the basal secretion of these three hormones in perfused pituitary cells. Most basal GH and PRL secretion is extracellular Ca^{2+} dependent. Application of tetrodotoxin (TTX), a specific voltage-gated Na^{+} channel blocker, does not alter the pattern of spontaneous GH, PRL or LH secretion. By contrast, application of the Ca^{2+} channel blockers, nifedipine and Cd^{2+}, inhibits basal GH and PRL secretion without affecting basal LH secretion [10]. These results indicate that the main fraction of spontaneous GH and PRL *in vitro* release reflects regulated, Ca^{2+} influx-dependent exocytosis.

The extracellular Ca^{2+} dependence of basal PRL and GH release, but not LH release, is consistent with findings that cultured somatotrophs [11], lactotrophs [12] and immortalized pituitary cells [13,14], in addition to *in situ* somatotrophs [15], spontaneously fire action potentials (APs), whereas most unstimulated gonadotrophs from male rats are quiescent [16]. However, basal gonadotropin secretion is also low in cells from female animals, even though they fire APs spontaneously [10]. A comparison of spontaneous electrical membrane activity in all three hormone-secreting cell types under identical recording conditions using the perforated patch, whole-cell configuration and pituitary cells from randomly cycling female rats is shown in Figure 1b. Most somatotrophs and lactotrophs fire Ca^{2+}-dependent bursts of APs with a frequency of ~0.3 Hz, and ~50% of the gonadotrophs exhibit spontaneous single AP firing with a frequency of 0.7 Hz [10].

In general, excitable cells secrete in a regulated manner through AP-driven Ca^{2+} influx and Ca^{2+}-dependent exocytosis. There are some differences in molecular mechanisms underlying exocytosis and recapture of synaptic and secretory vesicles [17]. In addition, at synapses a single AP and a train of single APs are sufficient to trigger secretion, whereas in neuroendocrine and endocrine cells prolonged depolarization is required to activate the exocytotic pathway [18]. The rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is needed for two steps in exocytosis: priming the vesicles to the site of release and fusion of vesicles with the plasma membrane [19]. Thus, AP-driven secretion depends on the $[Ca^{2+}]_i$ in the vicinity of the secretory vesicles. Consistent with this, it appears that the pattern of basal anterior pituitary hormone secretion is determined by differences in the ability of APs to increase $[Ca^{2+}]_i$ in these three cell types. As shown in Figure 1c, somatotrophs and lactotrophs

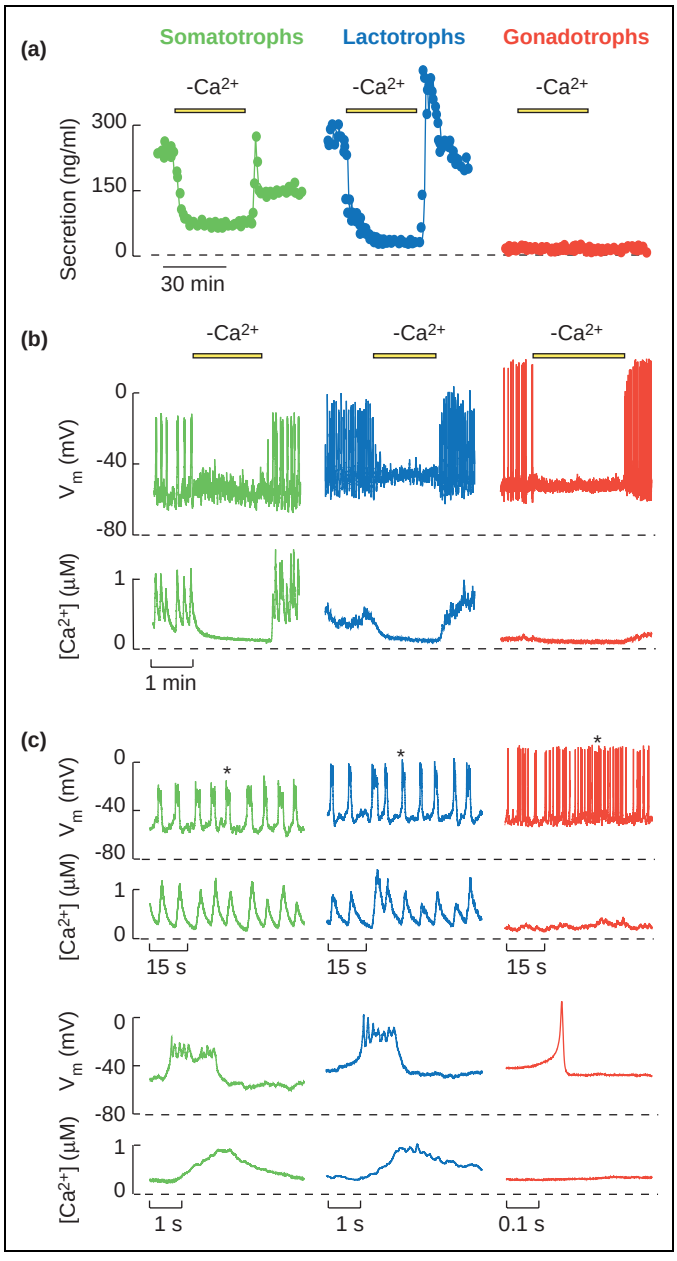

Figure 1. Characterization of electrical activity, calcium signaling and secretion in resting pituitary cells. **(a)** Effects of removal of extracellular Ca^{2+} on basal GH (left panel), PRL (central panel) and LH (right panel) release. Cells were transiently perfused with Ca^{2+}-deficient medium containing 100 μM EGTA (-Ca^{2+}). Secretion was normalized to account for differences in the sizes of somatotroph, lactotroph and gonadotroph populations in anterior pituitary cell preparations. **(b,c)** Simultaneous measurements of membrane potential (V_m) and $[Ca^{2+}]_i$ in single somatotrophs (left panels), lactotrophs (central panels), and gonadotrophs (right panels). (b) Extracellular Ca^{2+} dependence of electrical activity and Ca^{2+} signaling. (c) Plateau bursting versus single AP firing. Asterisks in the upper panel illustrate selected APs and corresponding Ca^{2+} transients, which are shown in the bottom traces on an extended timescale. (Notice the difference in the timescale for gonadotrophs.) Derived from [10].

generate slow resting membrane potential oscillations, with superimposed bursts of APs, with an averaged duration of seconds, accompanied by high-amplitude $[Ca^{2+}]_i$ signals that range from 0.3 μM to 1.2 μM. By contrast, gonadotrophs fire high-amplitude, single spikes with a duration of milliseconds, which generate low-amplitude $[Ca^{2+}]_i$ signals ranging from 20 nM to 70 nM. Immortalized pituitary cells can exhibit both single spike and plateau bursting patterns of firing [20].

Expression of depolarizing Na^+ and Ca^{2+} channels and secretion

In general, differences in the expression levels of ionic channels should underlie the cell type-specific patterns of APs, voltage-gated Ca^{2+} influx (VGCI) and hormone secretion. Previous studies indicated that pituitary cells express numerous plasma membrane channels, including TTX-sensitive Na^+ channels, voltage-sensitive Ca^{2+} channels (VGCCs), transient and delayed rectifying K^+ channels and multiple Ca^{2+}-sensitive K^+ channel subtypes. Both inactivating and non-inactivating VGCCs are found in rat gonadotrophs, somatotrophs and lactotrophs, and in immortalized pituitary cells. The inactivating Ca^{2+} current is mediated by the low-voltage-activated or T (transient)-type Ca^{2+} channel, whereas the non-inactivating Ca^{2+} current is mediated by dihydropyridine-sensitive [L (long lasting)-type] and dihydropyridine-insensitive, high-voltage-activated Ca^{2+} channels [2,3].

To exclude the possibility that differences in species, sex or hormonal status of the animals used, or cell culture and recording conditions, mask variations in the expression levels and the properties of ion channels, the amplitudes of various currents in somatotrophs, lactotrophs and gonadotrophs from randomly cyclic female rats were recently compared under identical culture and recording conditions [21]. This analysis revealed that lactotrophs and somatotrophs express lower levels of TTX-sensitive Na^+ channels than do gonadotrophs. However, as discussed above, these channels are not crucial for spontaneous electrical activity in dispersed pituitary cells, because TTX does not influence basal GH and PRL release in resting pituitary cells. All three cell types also express VGCCs. T-type current (labeled as peak I_{Ca} in Figure 2a,b) is more prominent in somatotrophs than in lactotrophs and gonadotrophs. In spontaneously active somatotrophs, these channels contribute to the generation of high-amplitude $[Ca^{2+}]_i$ transients [22], whereas their role in

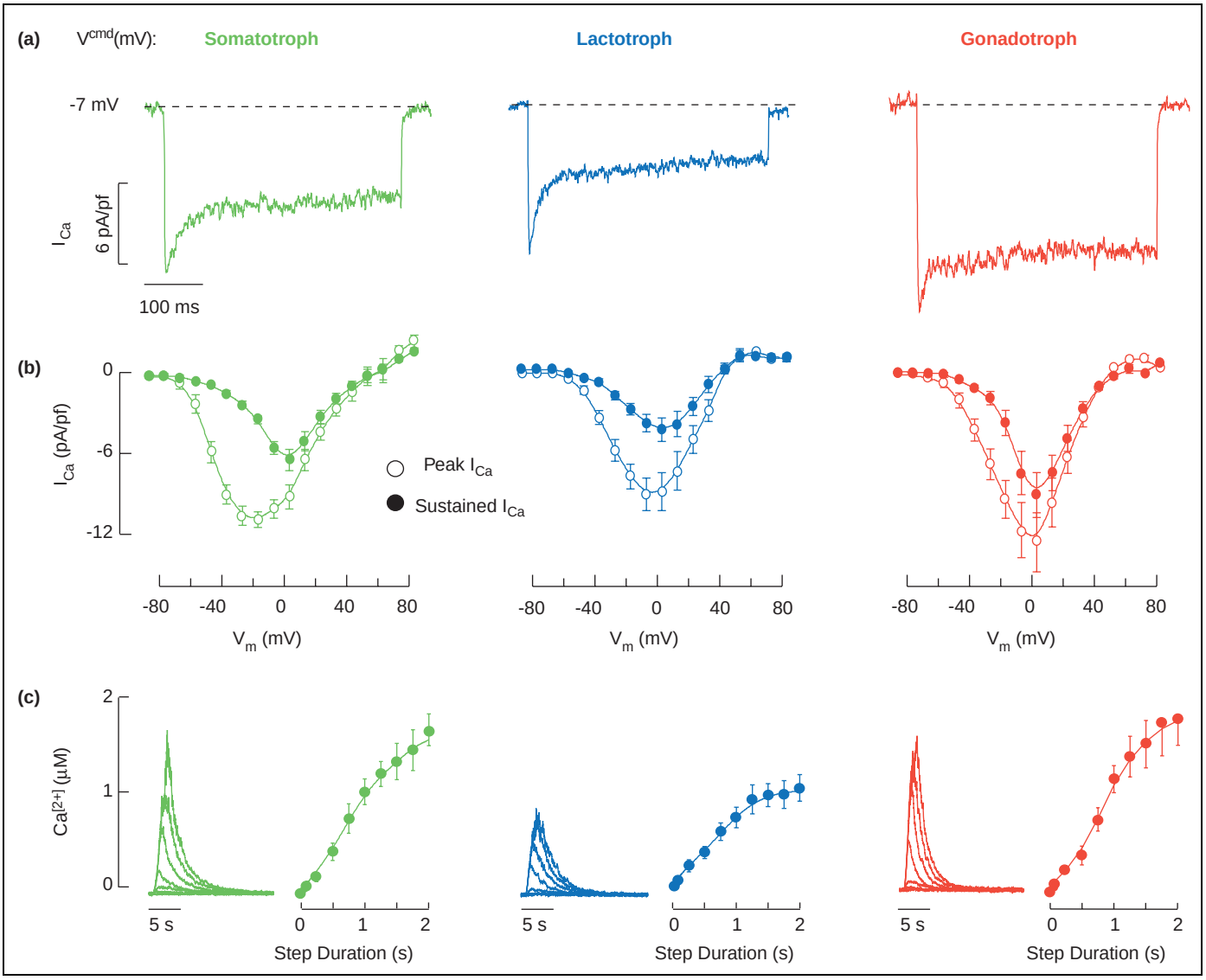

Figure 2. Voltage-gated Ca^{2+} channels in pituitary cells. **(a)** Representative voltage-gated Ca^{2+} current traces in somatotrophs, lactotrophs and gonadotrophs elicited by 400-ms voltage steps from a holding potential of −97 mV. **(b)** Current–voltage relation of the peak (open circles; 0–25 ms) and sustained (filled circles; 390–400 ms) voltage-gated Ca^{2+} current in all three cell types. **(c)** Depolarization-induced rise in $[Ca^{2+}]_i$ in somatotrophs, lactotrophs, and gonadotrophs. Cells were clamped at −90 mV and transiently (25 ms to 2 s) depolarized to −10 mV, and the accompanying increase in $[Ca^{2+}]_i$ was monitored. Left panels: typical profiles of $[Ca^{2+}]_i$ responses to depolarizing pulses of variable duration. Right panels: relationship between peak $[Ca^{2+}]_i$ responses and duration of depolarizing pulses. Derived from [10,21].

other native anterior pituitary cells is not well characterized. The current–voltage relationship of the sustained Ca^{2+} current (labeled as sustained I_{Ca} in Figure 2a,b) in all three cell types is similar, but the current density is higher in somatotrophs and gonadotrophs than in lactotrophs.

Three lines of evidence indicate that the duration of depolarization and accompanying VGCI, rather than the expression of VGCCs, is the major factor determining cell type-specific patterns of AP-driven Ca^{2+} signaling and secretion. As shown in Figure 2c, the peak amplitude in $[Ca^{2+}]_i$ increases progressively with lengthening duration of the depolarizing membrane potential step in all three cell types. A similar increase in the peak $[Ca^{2+}]_i$ occurs in somatotrophs and gonadotrophs. Furthermore, gonadotrophs, lactotrophs and somatotrophs show comparable $[Ca^{2+}]_i$ and secretory responses during steady-state depolarization of cells with 50 mM K^+, indicating that the secretory vesicles in gonadotrophs also respond to high-amplitude VGCI signals. Finally, a shift in the firing pattern of gonadotrophs from single spiking to plateau-bursting AP induced by BayK8644, an L-type Ca^{2+} channel activator, is sufficient to trigger LH secretion when accompanied by increased frequency of firing [10]. It is highly probable that an increase in the frequency of firing, without changing the pattern of APs, would also be sufficient to generate global Ca^{2+} signals and trigger secretion, but this relation has not yet been studied in gonadotrophs.

Cell type-specific expression of K^+ channels

K^+ channels are key determinants of cellular excitability and are subject to modulation by Ca^{2+} and other intracellular second messengers. All three cell types express slow inactivating K^+ channels, whereas functional expression of the transient K^+ channel is much higher in lactotrophs and gonadotrophs than in somatotrophs [21]. However, this difference is unlikely to underlie the cell type-specific firing pattern, because both lactotrophs and somatotrophs exhibit the plateau-bursting type of spontaneous electrical activity. The most dramatic differences are related to the expression of two types of Ca^{2+}-activated K^+ channels: BK (big) and SK (small). The presence of these channels has been demonstrated in immortalized anterior pituitary cells [23–25] and native intermediate pituitary cells [26]. Recent studies indicate that BK channels are also expressed in lactotrophs and somatotrophs, and that they are coupled to VGCI [21], whereas earlier studies established the expression of SK channels in gonadotrophs [27].

As shown in Figure 3 (upper panel), the two-step protocol (see Figure legend for details) applied to cells bathed in Ca^{2+}-containing, and subsequently in Ca^{2+}-deficient, medium indicates the presence of high-amplitude, VGCI-sensitive K^+ currents in somatotrophs and lactotrophs, but not in gonadotrophs (Figure 3a). Pharmacological identification of VGCI-activated K^+ currents is consistent with expression of the BK subtype of these channels in somatotrophs and lactotrophs. First, the specific BK channel blockers, iberiotoxin (IBTX) and paxilline, markedly reduce the K^+ current in these two cell types. Second, the BK channel activator, NS1619, increases K^+ current amplitude in somatotrophs and lactotrophs, but not in gonadotrophs. Third, unlike the BK channel blockers, the SK channel blocker apamin has no effect on the K^+ current elicited by the 100 ms Ca^{2+}-influx step in most of the cells examined [21]. Because the voltage-gated Ca^{2+} current density and the change in $[Ca^{2+}]_i$ elicited by the Ca^{2+}-influx step among the three cell types were comparable (Figure 2), it is reasonable to conclude that differences in the magnitude of the BK current among these cells reflect differences in their BK channel expression levels.

Paradoxical role of BK channels in controlling AP-driven Ca^{2+} entry

In immortalized GH pituitary cells, colocalization of BK channels with VGCCs facilitates spike repolarization, which limits AP-driven Ca^{2+} influx [28,29]. In these cells, BK channel activation can also influence the frequency of APs by slowing pacemaker depolarization [30]. On the basis of these results, it would be expected that the relatively high levels of BK channel expression in somatotrophs and lactotrophs should limit AP-driven Ca^{2+} influx in these cells more than in gonadotrophs, which express few of these channels. However, this hypothesis is inconsistent with the duration of the AP waveforms in the three cell types. In addition, both the amplitude and duration of the spontaneous, VGCI-dependent $[Ca^{2+}]_i$ transients are greater in somatotrophs and lactotrophs than in gonadotrophs (Figure 1c).

In somatotrophs pre-loaded with BAPTA/AM, a membrane-permeable Ca^{2+} buffer, the profile of the AP waveform is shifted from plateau bursting to single spiking (Figure 3e). Similar effects were seen during application of IBTX or paxilline (Figure 3f,g). These, and other results discussed in detail in [31], indicate that BK channel activation is needed to generate the sustained plateau potential that prolongs AP bursting duration and facilitates extracellular Ca^{2+} entry into somatotrophs. Unlike somatotrophs, gonadotrophs express few BK channels, fire single spikes that are not altered by BAPTA, IBTX or paxilline, and thus have a low capacity to drive Ca^{2+} entry. However, mathematical modeling of electrical activity in gonadotrophs indicated that these cells would also fire plateau-bursting-type APs if they expressed BK channels at levels comparable to somatotrophs and lactotrophs [31]. Thus, BK channels might have a somewhat paradoxical role in controlling AP-driven Ca^{2+} influx in these cells compared with other excitable cells, and their cell-specific expression accounts for the coupling of spontaneous electrical activity and hormone secretion in somatotrophs.

We propose a model for the generation of plateau bursting by BK channels in rat somatotrophs, with Ca^{2+} channels, BK channels and delayed rectifying K^+ channels playing the major roles in this process. According to this model, activation of VGCCs depolarizes cells and generates the first spike. The ensuing Ca^{2+} entry rapidly activates a fraction of the BK channels closely associated with VGCCs. This truncates the spike amplitude and thereby limits the magnitude of activation of the delayed

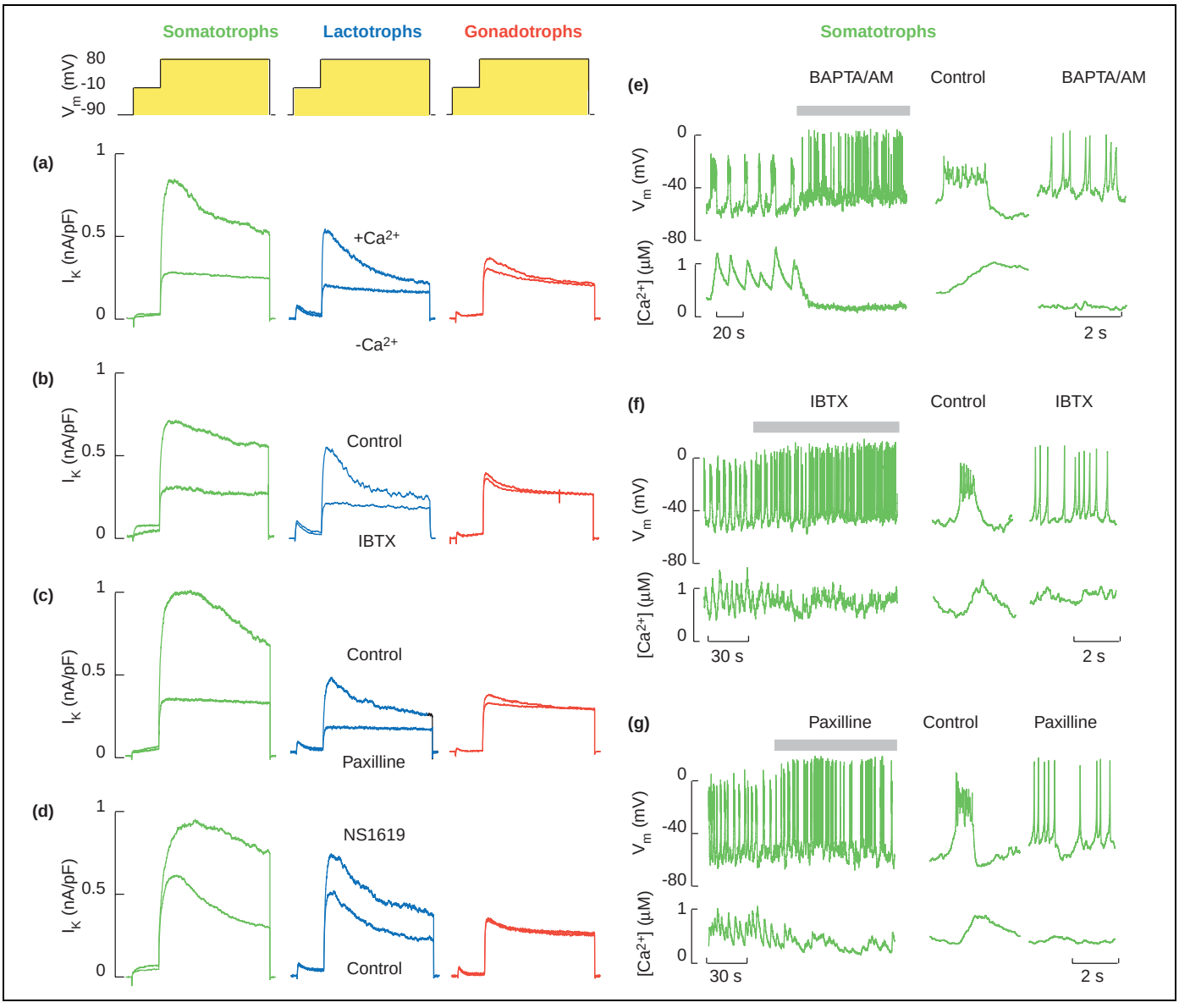

Figure 3. Pharmacological identification of BK channels in pituitary cells and their roles in control of AP waveforms. Top left panel: Two-step protocol composed of a 100-ms condition pulse to −10 mV (to activate voltage-gated Ca^{2+} influx), followed by a 500-ms test pulse to +80 mV (during which the K^+ current was monitored). Representative current traces showing the effects of removal of extracellular Ca^{2+} **(a)**, the addition of BK channel blockers IBTX **(b)** and paxilline **(c)**, and the addition of BK channel activator NS 1619 **(d)** on the K^+ current (I_K) in somatotrophs, lactotrophs and gonadotrophs. Simultaneous measurement of the membrane potential (V_m) and $[Ca^{2+}]_i$ in somatotrophs treated with BAPTA/AM **(e)**, IBTX **(f)** and paxilline **(g)**. Right panels: expanded timescale of APs and associated $[Ca^{2+}]_i$ signals. Derived from [21,31].

rectifying K^+ channels. In that scenario, the plateau potential results from the balance between the outward delayed rectifying K^+ channels and BK channels and the inward current through VGCCs. The generation of the plateau potential results in sustained VGCI and high-amplitude $[Ca^{2+}]_i$ transients. The eventual repolarization of the plateau potential might result from the recruitment of additional BK channels and/or activation of SK channels or Ca^{2+}-controlled Cl^+ channels.

Role of SK channels in plateau bursting in gonadotrophs

Native gonadotrophs can also generate a pattern of electrical activity resembling that seen in spontaneously active lactotrophs and somatotrophs, but only when the Ca^{2+}-mobilizing pathway is activated. As shown in Figure 4a, spontaneous firing of single APs in gonadotrophs is transiently abolished during application of GnRH, a Ca^{2+}-mobilizing agonist for these cells. This is followed by recovery of AP firing during sustained agonist stimulation, but such firing is interrupted by regular hyperpolarizing waves. The expanded timescale of sustained firing clearly indicates that the pattern of agonist-induced electrical activity resembles that seen in spontaneously active lactotrophs (Figure 4a,b). The role of SK channels in generating transient hyperpolarization waves is well established in GnRH-stimulated gonadotrophs from both neonatal [32] and adult rats [33]. Thus, in gonadotrophs these channels do not appear to be coupled to VGCI, but are colocalized with intracellular Ca^{2+} release sites.

It is important to stress that although the patterns of electrical activity in GnRH-stimulated gonadotrophs resemble spontaneous plateau-bursting activity in somatotrophs and lactotrophs, SK channels do not

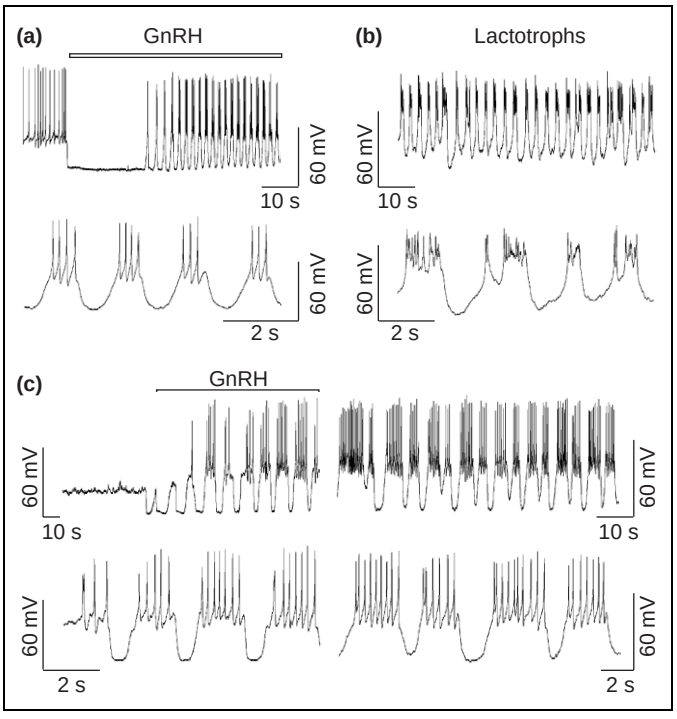

Figure 4. Comparison of plateau-bursting electrical activity in GnRH-stimulated gonadotrophs and resting lactotrophs. **(a)** Transition from single AP spiking to plateau bursting during GnRH application. **(b)** Spontaneous electrical activity in lactotrophs. **(c)** Sustained plateau bursting in gonadotrophs after removal of agonist. Horizontal bars indicate the duration of the GnRH pulse. Bottom traces in all panels: expanded timescales. Derived from [10,33,50].

facilitate VGCI; however, their deactivation leads to firing of APs of enhanced frequency compared with spontaneous firing (Figure 4a). This type of electrical activity occurs not only during GnRH action, but also for a prolonged period after removal of the agonist. Figure 4c illustrates a spontaneously silent cell, which begins to exhibit a plateau-bursting type of electrical activity during GnRH action that continues after removal of the agonist. It is well established that such a pattern of electrical activity promotes refilling of intracellular Ca^{2+} pools after removal of agonist [34].

Conclusions

Spontaneous (intrinsic) release of hormones from pituitary lactotrophs and somatotrophs was identified over 30 years ago, but the molecular mechanisms underlying this secretion remained largely uncharacterized. More recent studies have shown that electrical activity, and the associated extracellular Ca^{2+}-dependent exocytosis, underlie the intrinsic secretory activity of these cells. However, gonadotrophs also fire spontaneous extracellular Ca^{2+}-dependent APs, indicating that excitability per se is not sufficient for effective excitation–secretion coupling. The results also indicate that the pattern of spontaneous Ca^{2+} transients encodes cell type-specific basal hormone secretion. Extensive Ca^{2+} imaging studies, and more comparative simultaneous electrophysiological and Ca^{2+} measurements, revealed that somatotrophs and lactotrophs exhibit high-amplitude Ca^{2+} transients, whereas gonadotrophs exhibit no or only low-amplitude Ca^{2+} signals. It is also clear that cell type-specific expression of Ca^{2+}-activated K^+ channels plays an important role in shaping the pattern of AP waveforms. Pituitary lactotrophs and somatotrophs express the BK type of these channels, activation of which depends on Ca^{2+} influx driven by VGCI. In somatotrophs, or at least in a fraction of these cells, rapid BK channel activation by domain Ca^{2+} truncates the AP amplitude and thereby limits the participation of delayed rectifying K^+ channels in membrane repolarization, which leads to the generation of plateau-bursting activity and global Ca^{2+} signals (Figure 5). Conversely, pituitary gonadotrophs express relatively few BK channels and spontaneously fire single spikes with a low capacity to promote Ca^{2+} entry. However, gonadotrophs express SK channels, which are less sensitive to V_m, and the activation of these channels is not dependent on VGCI, but is coupled to agonist-induced Ca^{2+} release from intracellular stores. This provides for periodic firing of APs of higher frequency and VGCI, which sustains agonist-induced Ca^{2+} release and hormone secretion (Figure 5). See also Box 2.

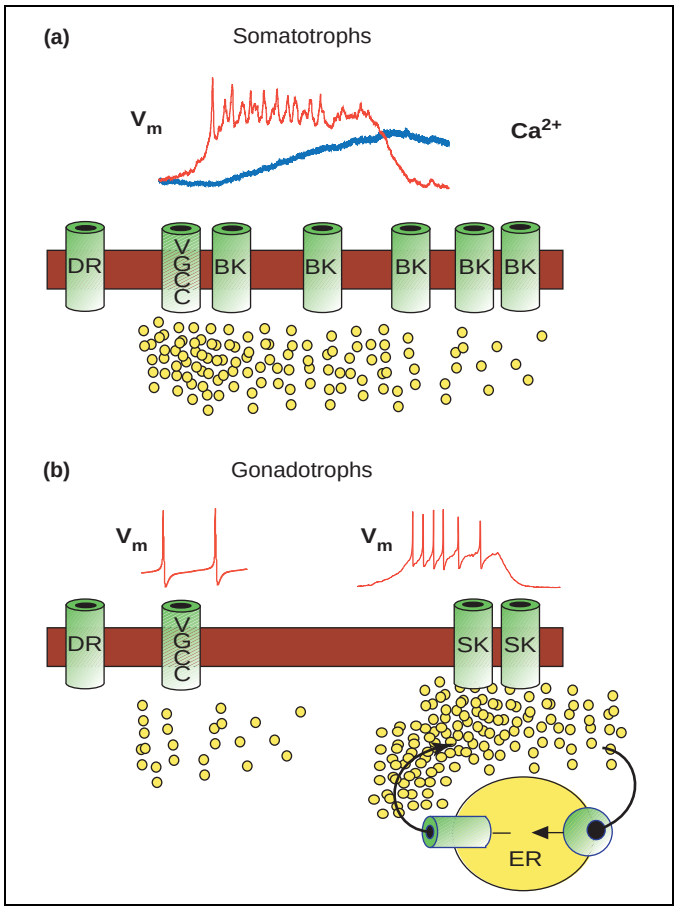

Figure 5. Schematic representation of the cell type-specific expression and regulation of Ca^{2+}-controlled K^+ channels. Upper panel: somatotrophs express BK channels, which are regulated by domain and global (bulk) Ca^{2+}, depending upon the distance between VGCCs and BK channels. V_m and Ca^{2+} traces are from a spontaneously active cell. DR, delayed rectifying K^+ channels. Bottom panel: gonadotrophs express SK channels that are regulated by agonist-induced and inositol (1,4,5)-trisphosphate-dependent Ca^{2+} release from the endoplasmic reticulum (ER). Activation of SK channels is followed by a shift from single AP firing to the plateau-bursting type of firing (V_m traces). In both panels, the small circles indicate intracellular Ca^{2+}.

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Box 2. Future directions

Despite the recent progress in understanding the spontaneous secretory activity of pituitary lactotrophs and somatotrophs, numerous uncertainties remain to be clarified. Lactotrophs and somatotrophs are highly heterogeneous subpopulations of pituitary cells with respect to their basal hormone release and responsiveness to agonists. Future single-cell electrophysiological, Ca^{2+} and secretory measurements should focus on characterizing such heterogeneity. For example, do all electrically active somatotrophs and lactotrophs exhibit plateau-bursting-type APs? What is the relationship between the frequency of APs and global Ca^{2+} signaling? What is the role of BK channels in lactotrophs, and what is the effect of blockade of BK and SK channels on hormone release? Why do spontaneous multiple APs not trigger high-amplitude global Ca^{2+} signals in gonadotrophs? The other line of questions is related to the mechanism of generating spontaneous APs. Which channels in these cells are involved in pacemaking activity? What is the relationship between Ca^{2+} driven by APs and other intracellular messengers in spontaneously active cells [41,42]? Are those messengers involved in control of pacemaking currents?

The continuous and high secretion of hormones by cultured lactotrophs and somatotrophs also raises many questions. The trafficking steps along the secretory and endocytotic pathways together should provide a balanced flow of membranes [19]. How is this achieved in somatotrophs and lactotrophs with such a high rate of spontaneous secretion? To which extent do the 'kiss and run' mechanism and the partial discharge of secretory vesicles [43,44] participate in this process? Does spontaneous VGCI-dependent regulation of adenylyl and guanylyl cyclase and protein kinase C activities in pituitary cells [45,46] influence the status of secretory vesicles and contribute to the secretory output? How is the phosphatidylinositol (4,5)-bisphosphate-dependent step in priming the secretory vesicles [47] achieved in spontaneously secreting somatotrophs and lactotrophs?

Differences in the patterns of spontaneous Ca^{2+} signaling among pituitary cells are also of potential clinical relevance. For example, there is a parallel between the patterns of Ca^{2+} signaling and the occurrence of pituitary adenomas; prolactinomas or mixed PRL/GH-secreting tumors are the most common pituitary adenomas, whereas gonadotrophs rarely produce adenomas [4]. This could indicate the relevance of cell type-specific Ca^{2+} signaling, in addition to other factors [48,49], to the control of cell proliferation. It is also known that pathological hyperprolactinema and subsequent infertility in humans is usually associated with dopamine delivery in pituitary, or the loss of dopamine inhibition. Because dopamine effectively blocks spontaneous electrical activity, the direct blockade of electrical activity could provide an alternative treatment.

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